Observations on Removal of Spines by Muricid Gastropods During Shell Growth

BY

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(3 Plates)

INTRODUCTION

While investigating the comparative functional morphology of the boring mechanism of muricacean and naticacean gastropods (Carriker, 1961), we had an opportunity to observe the behavior of many of the snails closely. Two species of Muricidae, *Murex brevifrons* Lamarck, 1822 and *Murex fulvescens* Sowerby, 1834, whose shells are ornamented with conspicuous varices and spines which run the breadth of the whorls, particularly drew our attention because of the possible role of the accessory boring organ (Carriker, 1969; Carriker & Van Zandt, 1972) in removal of the spines during shell growth.

As pointed out by Fretter & Graham (1962) and by Robertson (1965), in the process of spiral growth of the helicocone, the snail has to remove the older spines which come to lie along the inner lip of the aperture in order to make room for the new shell of the enlarging body whorl. If the spines were not removed, they would block the aperture and interfere with the movements of the snail in and out of its shell. In the case of *Murex brevifrons*, blockage would be almost complete; in that of *M. fulvescens*, only partial.

How snails remove these spines has not been reported. Since the molluscan mantle has the capacity to secrete shell as well as to remove what it has deposited (Fretter & Graham, 1962; Smith, 1969; Soliman, 1969), Fretter and Graham postulated that the spines and varices may be resorbed by the mantle. Robertson (1965) hypothesized that the spines may be broken or rasped off, or may be removed chemically, possibly by the same means that boring snails use to excavate holes in the shell of prey.

In *Murex brevifrons* and *M. fulvescens* a strong thick varix is formed at the edge of the outer lip of the body whorl at regular intervals. Each varix represents a resting period in the growth of the shell. The intervarices of the shell represent active periods of shell deposition. Little is known about how long it takes snails to lay down the spines or the shell between the varices. Abbott (1954) suggested less than two days for growth from one varix to the next in Muricidae.

This paper reports the results of observations in the laboratory on the deposition of shell and the removal of spines during growth of the body whorl from one varix to the next in *Murex brevifrons* and *M. fulvescens*.

ACKNOWLEDGMENTS

John W. Blake and Langley Wood assisted in the investigation. Dr. Juan A. Rivero had *Murex brevifrons* sent from Puerto Rico, and Paul Shave later collected snails of the same species in the same area. Dr. Virginia Peters collaborated in the use of the scanning electron microscope. The research in 1959 was supported in part by a grant from the U. S. Fish and Wildlife Service; that in 1968 and thereafter by Public Health Service Research Grant DE 01870 from the National Institute of Dental Research. This assistance is gratefully acknowledged. Systematics-Ecology Program Contribution No. 268.

MATERIALS AND METHODS

The specimens of *Murex brevifrons* were collected in the vicinity of Mayaguez, Puerto Rico, packed moist, and shipped by airmail. The snails survived the flights in good condition, fed actively, grew new shell, and deposited egg capsules in our running seawater trays. We collected specimens of *M. fulvescens* on a rock jetty off Shackleford Banks, North Carolina. These individuals likewise acclimatized readily to laboratory conditions. Both species were fed oysters, *Crassostrea virginica* (Gmelin, 1791).

Principal observations were made at the Institute of Fisheries Research, University of North Carolina, Morehead City, during the period August 1 to September 11, 1959. Salinity of the seawater ranged from 31 to 35‰ and the temperature from 24 to 29° C. Both species came from high salinity, partially sheltered habitats. Supplementary observations were made on individuals of *Murex brevifrons* at the Marine Biological Laboratory in 1968. Salinity of the seawater in running seawater trays was approximately 32‰ and the temperature ranged from 20 to 21° C. In both laboratories snails were illuminated by daylight coming through the laboratory windows, and during the early part of the evening by standard overhead artificial light.

For the observations in 1959, five *Murex brevifrons,* ranging in shell height from 64 to 95 mm, were placed in an aquarium 30 × 30 × 60 cm in size; and six *M. fulvescens,* ranging in shell height from 95 to 100 mm were set in a similar tank. A stream of seawater about 7 mm in diameter ran into and overflowed from each aquarium. The tanks had wooden ends and glass on two sides, and were placed with one glass side against the wall so that the back was partially shaded. Position and feeding activity of the snails were watched through the front glass and top of the aquaria. To examine the amount of deposition of new shell and progress in removal of spines, we lifted snails off the bottom without taking them out of the water. To photograph them, we removed them from the water for brief periods. Frequency of examinations varied from daily to weekly, depending upon the rate of shell deposition and proximity of the mantle edge to the base of the spines. We marked *M. brevifrons* by tying fine red plastic twine of various lengths to one of the large dorsal spines. Individual *M. fulvescens* were identified by shell form.

Microscopic examination of shell spines was done with a JEOLCO scanning electron microscope, JSM-U3, in Woods Hole, Massachusetts. Spines which were partly eroded at the base in living *Murex brevifrons* were broken off, rinsed in distilled water, dried quickly, coated with gold in a vacuum evaporator, and studied at magnifications ranging from 45 to 5000 ×.

OBSERVATIONS

During the varix periods (Figure *1*) when no shell growth was taking place, snails crawled actively about the aquaria, bored oysters, and fed voraciously through their boreholes. At the onset of the intervarix period and deposition of new shell, they crawled to the shaded parts of the aquaria and remained there with little or no movement, the foot firmly appressed to the substratum. They remained relatively stationary until the end of the intervarix period. During this period they were difficult to dislodge from the bottom. When placed at the lighted side of the aquaria, snails returned to the more shaded spots. Dislodgment from the bottom caused them to retract within the shell, generally drawing the operculum into the aperture. In the hope of watching the normal activity of the mantle edge at the base of the spines under water, we had placed squares of clean glass plate on the bottom of the aquaria; glass on which a snail might settle could then be upended to bring the aperture into view without disturbing the snail. Unfortunately, the snails avoided the glass plates.

We were able to follow closely the cleaning of the parietal area, removal of spines, and deposition of new shell by several *Murex brevifrons*. Observations on one individual (Table 1) were confirmed by those on others. During

Explanation of Figures *1* to *6*

Figure *1*: Apertural view of *Murex brevifrons* in the varix stage. To the snail's right (bottom of photograph) is the most recently formed varix and row of spines along the edge of the outer lip, and to the left (top) is an older varix and row of spines which will be removed as the body whorl grows 1.5 ×

Figure *2*: Apertural view of *Murex brevifrons* in an early phase of the intervarix stage. A thin layer of new shell is being deposited on the outer lip, and the base of the spines and shell surface on the inner lip are being eroded 2 ×

Figure *3*: Apertural view of *Murex brevifrons* at the end of the intervarix stage. The old row of spines has been completely removed. The thin new intervarix shell of the body whorl has been formed, and the lacy, convoluted, outturned flanges of the lip mark the location of the new varix and new row of spines 2 ×

Figure *4*: Apertural view of *Murex fulvescens* in an early phase of the intervarix stage (comparable to that in Figure *2*). Thin new shell is being added to the outer lip, and the base of the spines and parietal shell surface are being eroded 1.2 ×

Figure *5*: Close view of a spine of *Murex fulvescens* on inner lip of aperture whose base has been almost completely eroded. The parietal surface in front of and between the spines has also been eroded. Mid intervarix stage 4 ×

Figure *6*: Apertural view of *Murex fulvescens* at the beginning of the varix stage, photograph taken 10 days after that in Figure *4*. The row of new spines on the outer lip is almost completely formed, the inner lip has been coated with a glaze of new shell, and a zone of new shell, 11 mm in width, has been added to the outer lip 1.5 ×

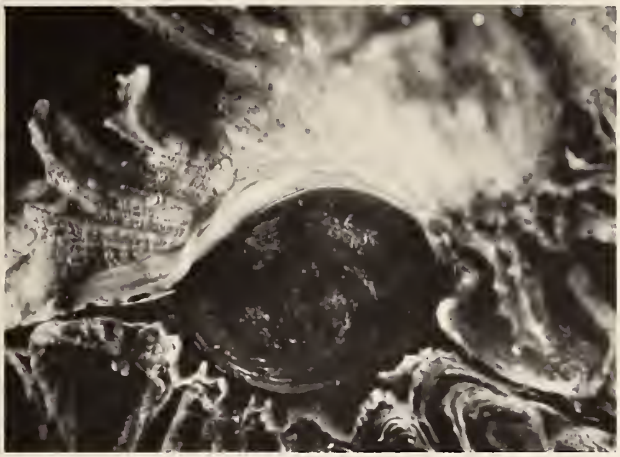

Figure 1

Figure 2

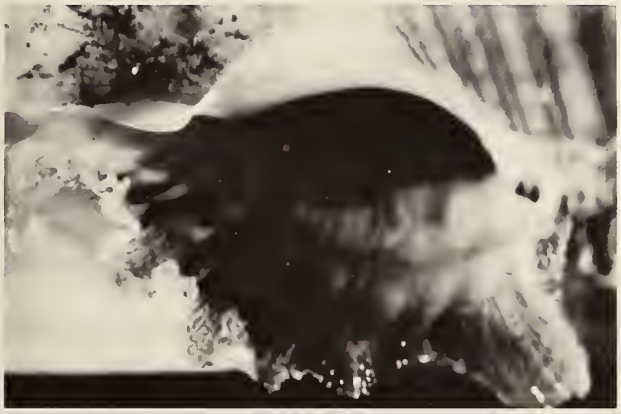

Figure 3

Figure 4

Figure 5

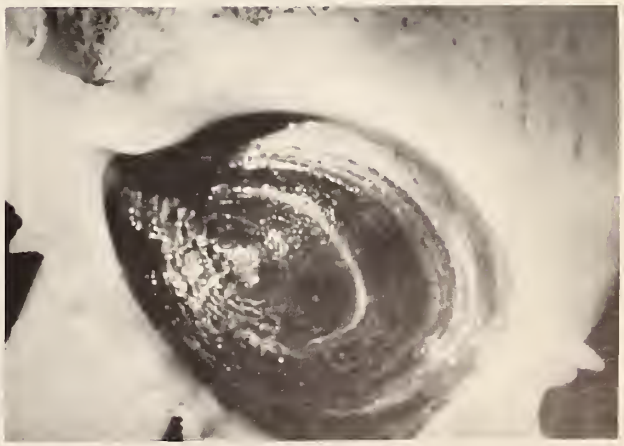

Figure 6

Table 1

Rate of deposition of shell on body whorl and removal of spines by *Murex brevifrons* during one intervarix period, August 7 to September 11

Days	Shell Height	Snail Activity	Width of New Shell Added to Outer Lip	Fate of Spines on Inner Lip
0	64 mm	End of feeding period	None	Spines intact (Figure *1*)
12		Inactive	Trace	Inner lip eroded to base of spines
18		Inactive	4 mm	Base of spines being eroded (Figure *2*)
31	69 mm	Inactive	26 mm	Spines removed and stumps covered with new parietal shell
35	74 mm	End of inactive period	30 mm, including new varix and flaring base of new spines	Position of removed spines now almost on the level with new varix at posterior base of aperture (Figure *3*)

the intervarix period which lasted about 35 days, this snail grew from 64 to 74 mm in shell height, and added a maximum width of 30 mm of new intervarix shell to the outer lip, most of the new varix, and the accompanying row of spines.

As accurately as we could determine, it took this snail about 8 to 10 days to remove a single row of spines. Incompletely removed spines showed conspicuous evidence of erosion at the base just above the level of attachment to the parietal area of the aperture and on the side facing the mantle edge (Figure *2*). The area of erosion was restricted to the parietal area and to the base of the spines. Spines were removed close to the surface of the inner lip so that after the new coat of shell had been deposited over the newly cleaned parietal area, there was no trace of them (Figure *3*). The freshly deposited intervarix shell on the outer lip was extremely thin (Figure *2*). During the following varix period this was thickened by deposition of more shell interiorly by the mantle, resulting in the characteristically thick valve of the species.

The manner and time for removal of spines by *Murex fulvescens* was approximately similar to that by *M. brevifrons* (Figures *4* to *6*). The snail in Figure *4*, for example, had deposited a rim of thin new shell 12 mm wide on the outer lip, and dissolution of the parietal area and the base of the spines had begun. Ten days later (Figure *6*) the spines had been completely removed and the parietal wall had been coated with a smooth layer of shell which completely hid the site of the original row of spines.

The advancing edge of erosion of the surface of the shell in the inner lip was more or less uniform up to the spines. As the base of the spines was attacked, areas of shell removal extended between the spines and slightly beyond (Figures *2, 4*). After the spines had been removed, the border of erosion again straightened.

Study by scanning electron microscopy of the partially eroded base of the spines of *Murex brevifrons* clearly revealed a delicately etched shell surface, and exposed a strikingly variegated pattern of complexly oriented shell prisms (Figures *7* to *10*; *11* to *14*). Figures *7* and *8* are low magnifications of the area of solution of the shell at the base of two spines. The topography of the eroded areas was divisible into an upper portion with conspicuous vertical striae, and a lower portion in which the striae were much less prominent and ran horizontally. Two prominent keels on each side of each spine separated the front from the sides.

No radular rasp marks were visible on the eroded surfaces of the spines. When rasping hard surfaces, muricid snails employ mainly the tricuspate rachidian teeth (Carriker & Van Zandt, 1972). The width of rachidian teeth in *Murex brevifrons* ranged from 200 to 300μ, and the distance between the points of adjacent cusps varied from 65 to 75μ. Thus had rasp marks been present, the interval between the individual cusp traces would have ranged between 65 and 75μ. At a magnification of 45$\times$ (Figures *7, 8*) the traces would have been about 3 mm apart; at a magnification of 100$\times$ (Figure *9*), about 7 mm apart.

At low magnifications (Figures *7, 8*) the striae, but for their branching, did resemble rasp marks; at higher magnifications (Figure *10*; Figures *11* to *13*), however, the striae resolved into differentially dissolved strata of shell prisms. The ridges of the vertical striae (Figures *10*; *11, 12*) were comprised of slender prisms whose long axes

came close to paralleling the general plane of dissolution, whereas the narrow depressions represented similar prisms whose ends abutted more nearly at right angles to the plane of dissolution. In the horizontal striae the difference in elevation between the layers of prisms was slight, and the long axes of the prisms in the ridges and the valleys, though appearing at approximately right angles to each other, formed nearly similar angles with the plane of dissolution (Figures *13, 14*).

DISCUSSION AND CONCLUSIONS

These observations demonstrated that removal by *Murex brevifrons* and *M. fulvescens* of spines obstructing the aperture during growth of the shell is done by chemical dissolution at the mantle edge. After the base is eroded through, the spines fall away. This conclusion is based on the pattern of the advancing edge of dissolution over the parietal area to, and past, the spines, the ultrastructural appearance of the eroded surface, and the absence of rasp marks on the eroded area. The remarkable capacity of the gastropod mantle to function both in shell dissolution and deposition is mentioned briefly by FRETTER & GRAHAM (1962). What portion of the complex mantle border secretes the dissolving substance, and what portion deposits shell, or whether the same tissue functions alternately in shell formation and shell dissolution, are not known. If the latter were the case, it would be interesting to speculate on the nature of the snail's integrating mechanism which brings this about, and what triggers the change from dissolution to deposition. Related aspects of the problem of shell deposition were treated by BEVELANDER & NAKAHARA (1970), DIGBY (1968), SALEUDDIN (1970), and WILBUR (1964).

Because of the active role of the radula in boring holes in shell of prey (CARRIKER & SMITH, 1969; CARRIKER & VAN ZANDT, 1972), we anticipated when the study was begun that the radula might be involved in removal of the spines. There is no apparent anatomical barrier to such a possibility as the proboscis of these snails is long enough to permit rasping around the inner lip of the aperture. Although we found no evidence of rasp marks on the eroded portion of the spines, even with the scanning electron microscope, the possibility may exist that we examined the snails at a time when only chemical activity was taking place. Since, however, the mantle edge dissolves the surface of the parietal area as well as the base of the spines, it is more likely, as our observations suggest, that the operation of cleaning the shell surface and removing obstructions as the body whorl enlarges is done entirely by the mantle. Furthermore, the hardness of the shell of these snails would suggest, as is the case with *Urosalpinx cinerea* (CARRIKER & VAN ZANDT, 1972), that radular cusps, if used, would have only minor impact on the surface of the spines anyway.

Examination with the scanning electron microscope of the dissolved surface of the spines revealed an unexpectedly complex organization of strata of shell prisms. Interdigitation of the strata in the spines undoubtedly contributes to the strength of these structures. MACCLINTOCK (1967) described the light microscopical structure of the shell of patelloid and bellerophontoid gastropods in detail, and illustrated crossed layers similar to those in the spines of *Murex brevifrons*.

Prisms whose long axes tended to parallel the surface of dissolution comprised the ridges of the striae, and those ending most nearly at right angles to the surface of dissolution formed the depressions. This pattern suggests that the sides of the prisms were slightly less soluble to the secretion of the mantle than the ends of the prisms. Differential dissolution may have resulted from the orientation of the molecules in the prisms, or from more protection afforded the sides of the prisms than the ends by the organic matrix. The matter needs experimental verification.

Whether the prominent keels on the base of the partially dissolved spines resulted from the way the mantle edge

Explanation of Figures 7 to *10*

Figure *7*: Scanning electron micrograph of the base of a spine in the mid intervarix stage of *Murex brevifrons* showing the three faces of the eroded region 45 ×

Figure *8*: Scanning electron micrograph of a second spine in the mid intervarix stage of the same *Murex brevifrons* showing the right, middle, and part of the left faces of erosion and the fracture surface where the spine was attached to the body whorl 45 ×

Figure *9*: The juncture on the middle face of the planes of erosion illustrated in Figure *8*. Vertical striae are at the top portion and the horizontal striae are at the bottom portion of the micrograph 100 ×

Figure *10*: Vertical striae shown in Figure *9*. Scanning electron micrograph 500 ×